cerning the specific mechanisms involved. However, it appears that amphetamine must have produced some long-lasting physiological change in the animals which alleviated fatigue, sustained motivation, or enhanced their ability to withstand stress.

Summary. Five groups of mice were trained to swim a maze in 4 trials and were given 6 test trials one week later. Group I was trained on saline and tested on saline (S-S); Group II was trained on saline and tested on amphetamine (S-A); Group III was both trained and tested on amphetamine (A-A); Group IV was trained on amphetamine and tested on saline (A-S), and Group V was injected with amphetamine one hour *after* training and was tested on saline (A after -S). The *test* data for trials 1 and 6 were then analyzed to determine whether the presence of the drug during training had a residual effect (learning effect) and/or if the presence of the drug during testing produced significant differences (performance effects). No learning effects were found on either trial, but, on the last trial, amphetamine significantly improved performance, and this effect outlasted the drug's presence in the central nervous system. It was hypothesized that amphetamine produced some persistent physiological change in the animals which maintained their performance.

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Stem Cell Replacement in Normal Thymus Grafts.* (29022)

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It has been postulated by some that thymic lymphocytes may be a special class of cell, distinct from other lymphocytes in the body. This implies the existence in the thymus of a permanent population of special thymic lymphoid stem cells, which by division could maintain the supply of thymic lymphocytes. However, evidence from the repopulation of the thymus in irradiated, bone marrow-injected animals(1,2) and of thymus grafts in thymectomized recipients(3) sug-

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TABLE I. Chromosome Analyses of Mitotic Cells in AKR Thymus Grafts in (AKR × T6)F1 Mice.

Days after grafting	No. of thymuses grafted	No. of grafts analyzed	No. of metaphases T6/+	No. of metaphases T6/−	Total No. of metaphases analyzed
3	6	1	0	2	2
6	12	4	4	103	107
7	15	5	16	164	180
11	24	7	39	323	362
13	4	2	36	80	116
14	16	3	86	116	202
15	4	1	66	30	96
16	4	4	633	0	633
17	9	3	247	19	266
20	12	7	527	2	529
26	4	4	414	0	414
27	4	2	200	0	200
31	9	7	680	0	680
33	4	2	248	0	248
37	2	2	200	0	200
38	4	3	204	0	204
39	5	4	303	0	303
40	7	4	400	0	400
102	1	1	3	0	3
Total	146	66	—	—	5145

gests that other lymphocytes can seed in the thymus and there behave as thymic lymphocytes.

To determine the situation in the normal animal, a study has been made of the origin of the dividing cells in normal AKR thymus grafts in normal young adult (AKR×T6)F1 hybrid mice. Chromosome data recorded earlier(4) have been extended and parallel cytological and histological analyses have been made of the developing thymus grafts.

Materials and methods. AKR and CBA/T6T6 mice were obtained from inbred colonies in this Institute. Six-week-old hybrid (AKR×T6)F1 mice (carrying one T6 marker chromosome in every mitotic cell) were grafted s.c. under nembutal anesthesia with one to 3 whole one-day-old AKR thymuses of the same sex. It has previously been shown(5) that the growth of thymus grafts is unaffected by the presence of other thymus grafts in the same animal. Donor thymuses were gently inserted in subcutaneous pockets either in the axillae or over the abdominal wall.

At intervals after grafting, host mice were injected i.p. with 0.25 ml of a 0.05% solution of Colcemide (CIBA). Ninety minutes later the thymus grafts were removed, cut into small fragments, bathed in 1% sodium citrate and incubated at 37°C for 20 minutes. Fragments were fixed in 60% acetic acid at room temperature for 1 hour, then squash preparations were made. Examination of the mitotic cells was made using phase contrast at × 900 magnifications. Consecutive, well-spread, metaphases were counted to ensure that all 40 chromosomes were present and the presence or absence of the T6/+ marker chromosome determined. Duplicate slides made from different portions of each graft were analyzed by two independent observers. In parallel experiments recipient mice were killed at intervals after grafting and the thymus grafts fixed, blocked, sectioned at 5 μ, and stained with Mayer's acid hematoxylin and eosin. Data from male and female grafts were similar and have been pooled in the results.

Results. Control thymus preparations from AKR mice were examined and all metaphases were found to lack chromosomes resembling the T6/+ chromosome (Fig. 1). One hundred and forty-six AKR thymuses were grafted for chromosome analysis between days 3 to 102 after grafting. Of these, 66 were satisfactory for analysis. A high percentage of the grafts examined in the first 2 weeks after grafting were too small to provide enough satisfactory metaphases for

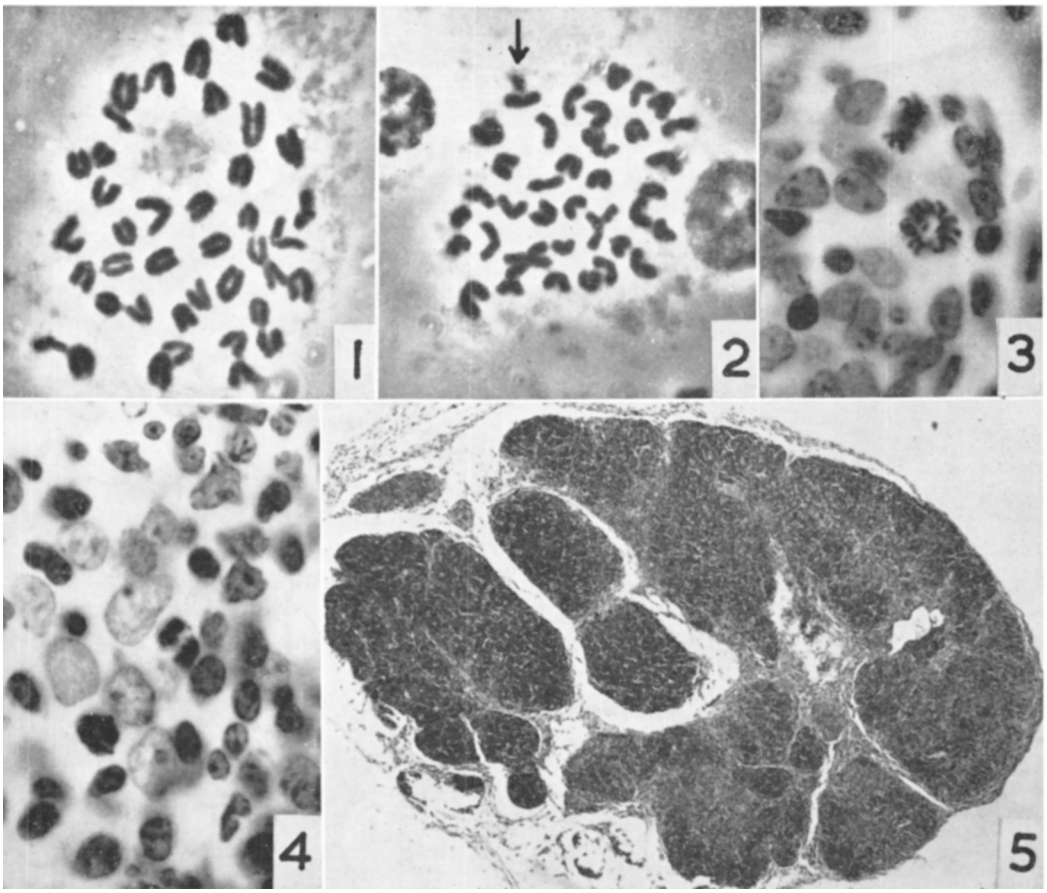

FIG. 1. Metaphase from an AKR thymus showing normal 40 chromosomes. Phase contrast × 1375.

FIG. 2. Metaphase from an AKR thymus graft in an (AKR × T6)F1 mouse showing T6/+ marker chromosome (arrow) indicating origin of cell from host. Phase contrast × 1375.

FIG. 3. Two epithelial or reticular cells in mitosis. Feulgen × 1400.

FIG. 4. Two lymphocytes in mitosis. Note size in relation to resting epithelial cell nuclei. Feulgen × 1400.

FIG. 5. AKR thymus, 8 days after grafting. Extensive lymphoid repopulation. 93% of mitoses donor-type. 77% of mitoses in lymphoid cells. H & E × 55.

analysis. Table I presents a summary of the graft analyses. Donor mitoses (lacking the T6 marker chromosome) predominated in grafts 3 to 14 days after grafting, but after this time mitoses of host origin rapidly increased, and in grafts older than 20 days, only host-type mitoses (bearing a T6 marker chromosome, Fig. 2) were found. This transition is shown more clearly in Fig. 6. Between days 3-20, some variation in the content of donor mitoses was observed between individual grafts of the same age, even between grafts in the same host animal.

Histologically, by day 4 after grafting widespread death of donor lymphocytes in the graft cortex had occurred. This led to collapse inwards of the organ around surviving islands of medullary epithelial cells and the reticular framework cells of the cortex. However, in none of the grafts were lymphocytes completely absent. In some grafts, a central necrotic area had developed, with surviving epithelial cell clusters and reticulum cells present in a cortical rind around the central necrotic area. By day 6, foci of lymphocytes had reappeared in most grafts. Some foci were located under the capsule but these showed little mitotic activity. In other

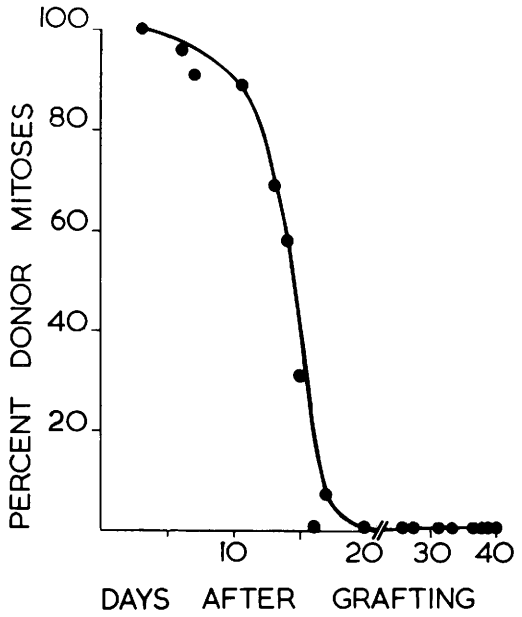

FIG. 6. Disappearance of donor-type mitoses in one-day-old AKR thymus grafts in (AKR × T6)F1 mice.

areas, mixed aggregates of lymphocytes and epithelial cells were present and in these mitotic activity was intense in both cell types. As the age and size of the grafts increased, lymphocyte aggregates became more extensive, and became localized in the peripheral regions of the graft. From day 8 these began to show the high mitotic activity typical of normal thymic tissue. Considerable variation occurred between individual grafts of the same age in the rate at which repopulation by lymphoid cells took place.

Mitotic cells were classified as (1) epithelial and/or reticular—long thin chromosomes, cell diameters $>10\ \mu$ (Fig. 3). (2) Lymphocyte—short condensed chromosomes, cell diameter $<6\ \mu$ (Fig. 4). (3) Intermediate—size of cells and shape of chromosomes intermediate between 1 and 2. Although proof was not possible, it is likely that the mitotic cells of the intermediate type were lymphocytes of a larger more primitive type than those of Type 2. This analysis revealed (Table II) that epithelial and/or reticular cell mitotic activity was intense in early grafts, accounting for some of the donor-type mitoses observed (both these donor cell types survived grafting). After day 18, mitotic activity in these cells virtually ceased. Lymphocyte mitotic activity was present even in the earliest grafts, and the frequency of lymphocyte mitoses increased steadily with the increasing size and lymphoid content of the grafts. Correlation of this data with that in Fig. 6 reveals that there was a temporary period in the life history of the grafts (between days 4-10) when donor-type lymphocytes were proliferating freely and increasing in total numbers. An example of such a graft is shown in Fig. 5. The disappearance of donor-type lymphocyte mitoses after day 18 cannot be accounted for by simple dilution by the increasing numbers of host-type lymphocytes.

A small group of grafts from adult, 8-week-old, donors was subjected to chromosome analysis. Thirty such grafts were analyzed

TABLE II. Cytological Analyses of Mitotic Cells in AKR Thymus Grafts in (AKR × T6)F1 Mice.*

Days after grafting	No. grafts analyzed	Mean graft wt (mg)	Mean percentage mitoses in: Epithelial or reticulum cells	Intermediate cells	Lymphocytes
4	6	3	36%	30%	34%
7	9	2	27	27	46
8	2	5	23	27	50
10	5	6	16	21	63
15	4	8	5	15	80
18	1	15	4	12	84
25	4	35	.5	7	92.5
42	4	40	.3	6	93.7

* Sample size. Days 4-8: All mitoses in 3 complete sections of whole graft. Days 10-42: All mitoses in 3, 70μ wide, zones across center of graft.

on days 18, 19, 22, 23, 24, 35, 36 and 41 after grafting. The percentage of host type mitoses was similar to that found in grafts from one-day-old donors. In an additional experiment, recipient (AKR×T6)F1 mice were thymectomized when aged 7 weeks, and one week later grafted with one-day-old AKR thymuses. Four grafts were examined 14 days after grafting and a mean percentage of 29% host mitoses found. In 3 grafts examined 21 days after grafting, 99.5% mitoses were host type.

Discussion. After grafting, the donor-type mitotic cells in normal thymus grafts in normal hosts were gradually completely replaced by mitotic cells of host origin. Since the lymphoid cell population of thymus grafts probably originates from local proliferative activity *in situ,* the present results indicate that most of the lymphoid cells in thymus grafts come, in time, to be derived from host cells.

A few lymphocytes in older thymus grafts may be long-lived survivors from the original donor cell population in the thymus at time of grafting, but if so these surviving cells must form a small percentage of the total population and apparently are incapable of mitotic activity in the grafts. It should be emphasized that in the first 2 weeks after grafting there was active proliferation of donor lymphocytes, but that this mitotic activity subsequently ceased.

These findings suggest that there may not be a permanent population of special lymphoid stem cells in the thymus, surviving for the life of the animal and continuing to give rise by mitosis to thymic lymphocytes. Instead it seems possible that the population of primitive cells in the thymus is continually replaced by immigrant cells of unknown type, entering from the circulation. Harris, Barnes and Ford (Ford, personal communication) using parabiotic CBA/CBAT6T6 mice have recently demonstrated a similar, but slower, entry of cells from the circulation into the pool of mitotic cells in the normal thymus.

Neither thymectomy of the adult host animal nor the use of adult rather than day-old thymus grafts materially affected the rate of replacement of the donor population of dividing lymphoid cells in thymus grafts by host cells.

Summary. After initial death of most donor lymphocytes in AKR thymus grafts in normal (AKR×T6)F1 mice, there was a temporary period during which active proliferation of surviving donor lymphocytes occurred. This population of dividing lymphocytes in the thymus grafts was subsequently completely replaced by host-type cells. The results suggest that thymic lymphoid stem cells are continuously replaced by cells entering the thymus from the circulation.

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Studies on Thiamine Absorption. (29023)

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In man, urinary output of thiamine as per cent of dose was shown to decrease as the dose was increased [see (1) for review]. Doses of thiamine up to 400 μg were readily absorbed by the rat(2), as based on urinary output, but larger doses were not(3). This suggested that proportionately less of the vitamin was being absorbed at the higher